PARTICLE ABSORPTION: SAMPLE FILTER PREPARATION AND ANALYSIS, QUANTITATIVE FILTER TECHNIQUE (QFT)

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Supplies Needed

Combusted 25mm Whatman Glass fiber GF/F filters (0.7 μm nominal pore size)
Glass filter cups and stems
Gloves
Forceps
Histoprep tissue capsules (Fisherbrand 29x6 mm, Cat no: 15-182-219; white)
Kim wipes
Liquid nitrogen (LN) dewar

Prior to field campaign

- Combust the appropriate amount of 25mm GFF filters at 450°C for 6 hours. Note the lot number. During analysis you will use the same lot of filters.
- Combust the glass filter cups. Do not combust the stems; they will be damaged.

Sample Collection and Storage

- Set up **glass** filter cups/stems with **combusted** GF/F filters.
- Measure the required seawater volume with a graduated cylinder and pour into appropriate-sized amber bottle. **Record** filter volume (Vf) in log sheet.
- Filter samples under low vacuum (5-7 psi).
- Repeat steps 1 through 3 until appropriate amount of color can be seen on the filter. For particulate absorption, **LIGHTER IS BETTER**. Only filter enough to see lite color, other wise you may either overload the sample or make extraction of chlorophyll more difficult.
- When the appropriate volume has been filtered, rinse the filter cup with 0.2 um filtered seawater. Do not let filter run dry under vacuum. Close valve when last few milliliters (ml) are running through the filter.
- Remove filter from stem with forceps. Place filter into a CLEARLY pre-labeled histoprep and store in LN. Samples can be stored for a longer term at -80°C.

Sample Analysis

INSTRUMENT: Perkin Elmer Lambda 35 UV/Vis Spectrophotometer
S/N 101N7060404

The Lambda 35 is a double beam UV-Visible spectrophotometer from Perkin Elmer, packing pre-aligned Tungsten and Deuterium Lamps. It has a wavelength range of 190-1100nm and a bandwidth range of 0.5 to 4nm.

Artificial Seawater (ASW) was used to maintain the moisture of the filters. The recipe was prepared using a modified version of Aquil Medium (Sunda et al., 2005). The recipe can found on the Provasoli-Guillard NCMA website under "Algal Medium Recipes." For this application, addition of the macronutrients, trace metals and vitamins is unnecessary.

a. Instrument Calibration and Maintenance

Internal wavelength accuracy and baseline stability tests are performed by the user before each use. A Holmium Oxide standard test is also performed to check wavelength accuracy. Currently, a service contract with Perkin Elmer exists to perform preventative maintenance or other services.

b. Reference Blank spectra

The method described based on Roesler (1998). An ESCO metallic coated Neutral Density filter (D=1.0; S51000) is kept in the reference beam in place of a GF/F filter to provide balance between the sample and reference beams, which allows for the transmission of 10% of the reference beam (comparable to transmission allowed by a GF/F filter). The neutral density filter is made from fused quartz, which allows for controlled attenuation in the UV. Blank filters are soaked in 0.2µm-filtered ASW for at least 30 minutes. Scans are performed between 300-800nm with a 2nm Slit Band Width (SBW), 0.1nm data interval and 120nm per minute scan speed.

c. Spectrophotometric Measurement Procedure

- Warm up the spectrophotometer for 30 minutes.
- Perform instrument performance tests.
- Baseline the system using air. Perform an air scan to assess the stability of the system. The scan (instrument noise) should measure ~0.000 absorbance units ±0.005. If not, system should be baselined again.
- Place the neutral density filter on the source side of the reference beam window using black electrical tape. Baseline the system is again, with air in the sample beam.
- Place a moistened blank GF/F filter on the detector side of the sample beam window and baseline the system again.
- Immediately following the baseline, without removing the blank filter, peform a sample scan of the blank filter. The scan should be flat. If the scan is not flat, then the system is baselined again. **This particular system is noisy in the UV (~300-375nm).** Rotate the filter 90 degrees and scan again.
- Scan moistened blank filters (~3-5) periodically throughout the analysis day to monitor instrument drift.
- For samples, place three to four drops of artificial seawater into a petri dish. Place the sample filter biomass up onto the water droplet. Allow the sample filter to thaw for 5 minutes before measurement. Cover the petri dish with the lid and foil to protect from the light.

- Place the sample filter on detector side of sample beam and measured three times at 0, 90 and 180 degrees.
- Measure the diameter (Df) of the biomass with calipers (Fisher Scientific Digital Caliper, model # 14-648-17)

Methanol Extraction method for de-pigmented particle absorption
The extraction protocol is based on Kishino et al. (1985).

- Place the sample filter on the glass filtration system.
- Gently add approximately 10-20ml of 95% methanol/5% ultrapure water to the filter cup and immediately filter at 5-7 psi.
- After the first 10-20 ml are filtered through, close the valve and add another 20 ml to the filter cup.
- Allow the sample to soak for AT LEAST 20 minutes. Cover the filter cup to prevent debris from contaminating the sample.
- Filter through and add another 20 ml methanol to the cup.
- Allow the sample to soak for at least another 20 minutes.
- After extraction, filter the last 20 ml of methanol through, and rinse the filter with 20 ml of ASW. DO NOT allow the filter to dry.
- Scan the moistened, extracted filter again using the protocol described in the *Spectrophotometric Measurement Procedure* section.
- If complete extraction of Chlorophyll is not attained, repeat the above extraction steps.

Data processing
- Mean of three a_p and a_d scans is calculated
- Blank scan closest to the sample scan is subtracted across spectra from the mean a_p and a_d scans (OD_f; if there is instrument drift).
- Absorption coefficient is calculated using the following equation

$a_p(\lambda) = [2.303*100/\beta*pathlength]*[OD_f(\lambda)-OD_{null}]$
OD_{null} = absorbance at 750nm

Pathlength = Vf (cm^3)/area of filter (cm^2)
Area of filter = $\pi*((Df/10)/2))^2 = \pi r^2$
Diameter was divided by **10 to convert mm to cm and by 2 to get radius**
$\beta = 2$ (Roesler, 1998)

To calculate spectral absorption of phytoplankton:
$a_{ph} = a_p - a_d$

Data reporting

Each SeaBASS submission of a_p scans will include the following:
- Blank-corrected raw absorbance of both a_p and a_d

- Standard deviation of rotation scans for both a_p and a_d
- Absorption coefficient calculations for each replicate (where applicable) for a_p, a_d and a_{ph}
- Standard deviation of absorbance of all blank filters scanned throughout the analysis period

Reporting Notation

abs_ap = raw total absorbance with blank subtracted (no null correction)
abs_ap_sd = standard deviation of the filter rotations
abs_ad = raw Ad absorbance with blank subtracted (no null correction)
abs_sd = standard deviation of the filter rotations
ap = absorption coefficient of total particles
ad = absorption coefficient of depigmented particles
aph = absorption coefficient of phytoplankton
abs_blank_sd = standard deviation of filter blanks

REFERENCES

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